International Journal of Medicine and Pharmaceutical Sciences (IJMPS)
ISSN(P): 2250-0049; ISSN(E): 2321-0095
Vol. 3, Issue 5, Dec 2013, 1-10

TRANS STELLAR
•Journal Publications •Research Consultancy

ANTIVIRAL PROPERTIES OF CYANOBACTERIUM, *SPIRULINA PLATENSIS*-A REVIEW

RANJANI RAMAKRISHNAN

Assistant Professor, Department of Virology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

ABSTRACT

Virus means poison and harmful to all forms of life. Viruses are acellular and obligate intracellular parasite cause viral infection in host cell. Viral diseases are observed in all living organisms including plant, animal and human. Carcinogenic viruses and other disease causative viruses including Chickengunya virus Dengue virus, SARS-CoV, HIV, Swine flu, Influenza virus, Herpes simplex virus, Human papilloma virus, oncorna viruses such as HTLV I and HTLV II are most important viruses spread disease worldwide. Some viruses are emerging, infectious, contagious. Prevention of virus transmission is important and is possible some extent by taking some important measures like creating awareness on transmission and pathology. Viruses are spread widely by many factors like environmental factors, contaminated, water, food, infected vector, person to person and to animals.

Resistance to the drug by host and virus is becoming one of the major problem in treatment with chemical drugs. To overcome this, one of the alternative ways is identifying biological compounds in the development of antiviral drugs and they are natural products especially from plants and algae. Natural products are effective against many viruses and act as antiviral drugs. Therefore, there is a need to focus on antiviral activities of natural products identified from plants to provide safe, lowest and effective drugs to control viral diseases of human. In the present study reviewed some of the important studies on the antiviral activities of microalgae *Spirulina platensis.*

KEYWORDS: *Spirulina platensis,* Antiviral, Virus, Phycocyanin, Polysaccharides, Ca-SP

INTRODUCTION

Human disease- causative infectious organisms or pathogens and way of spread from one person to person, animals, vector and environmental conditions (Table-1) was reported clearly (Louise *et al*., 2001; Cleverland *et al.*, 2001; WHO, 1996; 2013). Early detection of emerging infectious diseases and outbreaks in human and animal is crucial to the effective surveillance (Jo Halliday et al., 2013). Microbes both bacteria and virus lowers the quality of life and environment. Many emerging viruses cause disease in human and also in animals. Humans infecting virus species are nearly 219 and cause disease (Mark Whoolhouse *et al*., 2012). International Committee on Taxonomy of Viruses (ICTV) updated the list of viruses. Many emerging disease in human and also in animals caused by pathogens, microbes including viruses spread worldwide (Figure 1).

Table 1: Human Disease- Causative Infectious Organisms and Consisted of Pathogens and Way of Spread

S No	Infectious Pathogens: Total No: 1415 Species	No. Sp.S	Among These		Transmitted & Pathogenic to	Pathogen Transmission	
						Way of Spread	%
1	Bacteria and rickettsia	538	zoonotic	868 (61%)	animals and humans	Person to-person	65
2	Fungi	307	Emerging	175	human	food/water/soil	22
3	Helminths	287	(Louise *et al*., 2001)			insect/vector	13
			1415 Species		humans		

Table 1: Contd.,

4	Viruses and prions	217	374 Sp.s	domestic carnivores	directly from animal	<1
5	Protozoa	66	616 Sp.s	Livestock	(WHO, 1996).	
6 Ref	(Louise *et al*., 2001).		(Cleverland *et al*., 2001).		Food/insects environment (WHO, 2013).	

a) Aedes Aegypti **b) Aedes Albopictus**

Figure 1: Mosquito Vectors of Viruses (CDC:www.cdc.gov)

Transmission of disease causative agents by vectors especially mosquitoes contribute more. For example, Chikungunya virus (CHIKV), Dengue virus (DENV: Kazuya *et al.*, 2013) and many other disease causative pathogens are carried by infected mosquitoes to healthy person and avoiding mosquito bites is the key to avoid chikungunya, Dengue fever and spread of many diseases.

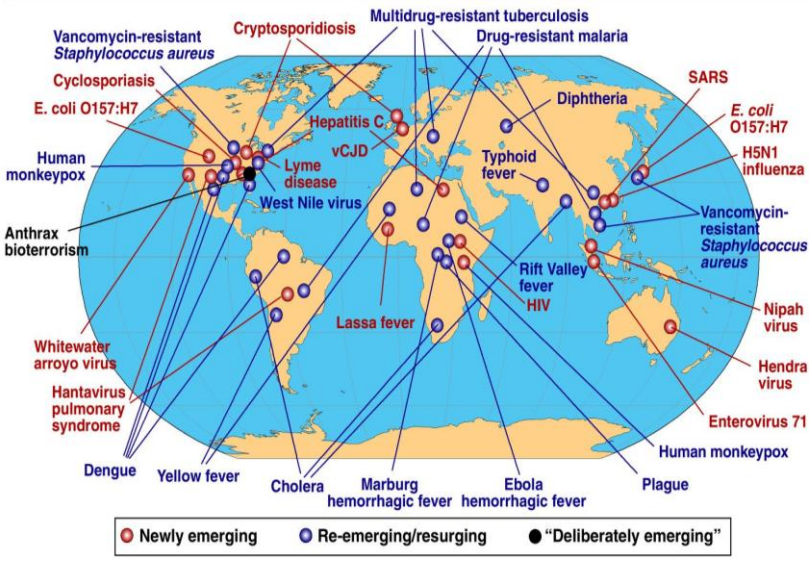

Figure 2: Emerging and Re-Emerging Infectious Diseases of Microbial Organisms: Examples (Clifton, & Lacy (2007)

People with a deficiency or an excess intake of nutrients must be balanced and need to lead healthy life (Gardner and Halwell, 2000). United Nations sources says that malnutrition and lack of key vitamins and minerals leading to impairment of physical and intellectual development (D.von der Weid, 2000; UNICEF, 2012). Food production, fresh water supply, bioenergy and adaptation to climate change are an essential to meet global requirements (FAO, 2009; 2012). This may be achieved to some extent from blue green algae, including *Spirulina platensis.* The results showed that *Spirulina platensis* is a good food with therapeutic agents.

In addition to *Spirulina* and its potential health effects, such as antibacterial, antioxidant, anticancer, immunomodulation and antiviral activities, some of the other positive effects also observed against some disorders like malnutrition, diabetes, hyperlipidemia, inflammatory allergic reactions, obesity, anemia, heavy metal/chemical-induced

toxicity and radiation damage but further larger trials are essential (Falquet, 1997; Belay, 2002; Gershwin and Belay, 2008; Karkos *et al.*, 2008; Archana Kulshestha *et al.*, 2008; Henrikson, 2009; Capelli and Cysewski, 2010; Deng and Chow, 2010; Soheili and Khosravi-Darani, 2011; Theodore G. Sotiroudis & Georgios T. Sotiroudis, 2013; Rakhi Bajpai Dixit & Suseela, 2013). Olivier Pignolet, *et al.,* (2013) Studied about antiviral properties of Cyanobacteria

Spirulina contains highest protein, essential amino acids, carbohydrates, essential fatty acids, minerals, vitamins, pigments. The three major bioactive components of *Spirulina*, biliprotein pigment Phycocyanin, sulfated polysaccharides and gamma linolenic acid play significant roles in improving human body functions. New experimental evidences support immunomodulation, potential health benefits and antiviral effects of *Spirulina* supplementation are mainly due to its chemical composition (Theodore G. Sotiroudis and Georgios T. Sotiroudis, 2013).

Antiviral Properties

The discovery of potent antiviral compounds extracts from blue green algae, including *Spirulina platensis* studied by researchers at the National Cancer Institute (NCI), USA. The NCI research group screened for antiviral compounds. They screened about 600 strains of cultured cyanobacteria representing some 300 species and 10% of the cultures produced substances caused significant antiviral effect by reducing cytopathic effects induced by viral infections (Patterson *et al.*, 1989). The potent virus inhibitors against several enveloped viruses are acidic polysaccharides such as calcium spirulan (Ca-SP) from *Spirulina platensis* and also other microalgae compounds like nostoflan from *Nostoc flagelliforme*, and a fucoidan from the sporophyll of *Undaria pinnatifida* (Hayashi, 2008). The acidic polysaccharide named as nostoflan of *Nostoc flagelliforme* exhibits potent virucidal activity against herpes simplex virus-1 (Kenji, *et al.* (2005). The synergistic antiviral effects on influenza A virus was confirmed *in vitro* as well as *in vivo* by administering compounds such as fucoidan or nostoflan with oseltamivir phosphate (Hayashi, 2008). *Microcystis ichthyoblabe* contains an ichthyopeptins A and B, two cyclic depsipeptides show antiviral activity against influenza A virus (Zainuddin, *et al.*, 2007).

Table 2: Antiviral Properties of Cyanobacterium, *Spirulina platensis*: Examples

S. No	Name of the Virus	Mechanism Involved	Compound Name	Author & Year	System
1	HSV -1	inhibited replication of HSV -1 and prolongs survival time of infected hamster	Water extract	Hayashi *et al.*,1993	Hamster
2	HCMV, measles virus, mumps virus, HIV-1, influenza virus, HSV -1	Inhibits replication of several enveloped viruses	Ca-SP (calcium spirulan)	Hayashi *et al.*,1996	*In vitro*
3	Viruses like HIV-1,HSV-1	Anti viral	Ca-SP	Hayashi *et al.*,1996	*In vitro*
4	HIV -1	• Inhibits replication of HIV-1 in human T-cell lines, PBMC • Inactivates HIV-1 infectivity	Aqueous extract	Ayehunie *et al.*, 1998	*In vitro*
5	Enterovirus 71	Delays viral RVA synthesis; activates apoptosis	allophycocyanin	Shih *et al.*, 2003	*In vitro*
6	HSV-1	Anti HSV-1	sulphoquinovosyl diacylglycerol	Chirasuwan *et al.*,2007;2009	*In vitro*
7	EBV	antiviral	Spirulina	Kok *et al*, 2011	*In vitro*
8	Many enveloped viruses & HCV (hepatitis C)	Activates Interferon gamma; Blocks virus absorption	Spirulina supplement	Yakoot & Salem, 2012	*Clinical trial*

Table 2: Contd.,

9	Adenovirus type 40, HIV-1, HIV-2, influenza virus, HSV -1	Antiviral	Aqueous & methonol extracts, Sulfated polysaccharides	Singh *et al.,* 2011 Sayda *et al*., 2012	*In vitro*
10	HIV	Immunostimulant	*Spirulina* supplement	Simpore *et al*, 2005Theodore G. Sotiroudis & Georgios T. Sotiroudis (2013).	*Human*

The aqueous extract and methanol extract of *Arthrospira maxima* showed antimicrobial- antibacterial activity against many bacterial organisms tested (Medina Jaritz *et al*., 2011).No inhibition was observed for poliovirus-1 subacute sclerosing panencephalitis virus (SSPE), measles virus, vesicular stomatitis virus (VSV) and rotavirus SA-11. Herpesvirus infection was inhibited by inhibiting virus adsorption and penetration to the host cell (beginning of the viral cycle). The *S. maxima* extracts prepared from methanol-water (3:1) exhibits highest antiviral activity was detected (Hernández-Corona *et al.,* 2002). The sulfated polysaccharide, calcium spirulan (Ca-SP) isolated from a hot water extract of *Spirulina platensis* displays anticancer activity. The calcium spirulan (Ca-SP) consists of sulfate, calcium, glucose, fructose, mannose, galactose, rhamnose, ribose, xylose, glucuronic acid and galacturonic acid (Hayashi *et al*., 1996). The extracts of cyanobacteria contains sulfated polysaccharides that prevents virus-cell attachment and fusion with host cells. The inhibition of the fusion between uninfected CD4+ lymphocytes and HIV-infected cells and thus greatly enhances viral infectivity (Feldmann *et al*., 1999; Rahul Kunwar Singh *et al*.,2011).

The water extract of *Spirulina platensis* inhibited the Herpes simplex virus type-1 (HSV-1) replication in HeLa cells (*in vitro*). The extract is not virucidal but interferes with the virus entry into host cells (Hiyashi *et al*., 1993). The calcium Spirulan (Ca-Sp) a sulfated polysaccharide isolated from *Spirulina platensis* inhibits many virus replications and exhibits antiviral activity against the HSV-1, influenza virus, Human cytomegalovirus (HVMV), mumps virus, measles virus and human immunodeficiency virus type 1 (HIV-1) (Hayashi *et al*., 1996) Spirulina acts as an antiviral agent (Armida Zúñiga-Estrada *et al.,* 2007; Simpore *et al.*, 2005).

The *in vitro* replication of HIV-I is inhibited significantly by the aqueous extract of *Spirulina platensis* by using human T-cell lines, peripheral blood mononuclear cells (PBMC) and Langerhans, blood mono nuclear cells The aqueous extracts of *S. platensis* possess antiretroviral activity (Ayenunie et al.,1998). The studies on anti –HIV activity and anti-HSV-1 activity of Ca-Sp was observed by inhibiting syncytium formation induced by HIV and HSV-1 plaque yield reduction respectively (Table 2). The Ca-SP of *S. platensis* is a potent antiviral agent against both HSV-1 and HIV-1 and Ca-SP can be a therapeutic drug (Hayashi *et al*., 1996).

The inhibitory effects on the herpes simplex virus type 1 (HSV -1) replication by performing structural modification of calcium spirulan (Ca- SP), a sulfated polysaccharide from Spirulina platensis (Lee *et al.,* 2001). The study made by Gorobets *et al*., (2002) revealed that the addition of *S. platensis* on bacteriophage T4 (bacterial virus) produced an inhibiting or stimulating effect on the reproduction of the bacteriophage in *Escherichia coli* B cells. The biological activities of algal sulphated polysaccharides was studied extensively (Witvrouw & De Clerq, 1997; Zvyagimtseva *et al.*, 2000). The purified allophycocyanin of spirulina platensis exbhited anti- enterovirus 71 activity and neutralized the cytopathic effects induced by enterovirus 71. The allophycocyanin delays viral RNA synthesis and activates apoptosis in both human rhabdomyosarcoma cells and Afrin green monkey kidney cells (Shih *et al.*, 2003). The white spot syndrome caused by white spot syndrome virus (WSSV) in black tiger shrimp (*Penaeus monodon*) and thus causes economic loss.

The effect of a crude extract from *Spirulina platensis* on white spot syndrome virus (WSSV) both *in vitro* and in *Penaeus monodon*.and inactivated WSSV significantly (Hemtanon *et al*., 2005).

The antiviral activity studied by a microplate inhibition assay using several viruses. The hot water extract of *Spirulina maxima* inhibits many viruses including herpes simplex virus type 2 (HSV-2), HSV-1, human cytomegalovirus (HCMV) and Pseudorabies virus (PRV) (Hernández-Corona *et al.,* 2002). In Japan and Korea most people eat seaweed daily. The consumption of *Spirulina,* ranges from 3-13 g daily in Asia and Africa. The prevention of HIV infection and suppression of viral particles in infected ones was observed by regular consumption of dietary algae (Teas *et al.,* 2004).

The supplement of *Spirulina platensis* for undernourished children seems to normalizes anaemia and causes weight gain in HIV-infected children, and even in HIV-negative undernourished children very quickly (Simpore *et al*., 2005). The Ca-SP targets viral absorption/penetration and some replication stages of progeny viruses (Hayashi, 2008). And also Ca-SP showed potent and broad-spectrum of antiviral activity against HIV-1, HIV-2, influenza and a series of other enveloped viruses (Feldmann *et al*., 1999; Rahul Kunwar Singh *et al*.,2011). Kazuya et al., (2013) studied inhibition of entry of Dengue virus by carbohydrate inhibitors.

The potent inhibitor of NADPH oxidase enzyme is chromophore -phycocyanobilin have versatile potential in the prevention and therapy of various diseases (McCarty, 2007; McCarty *et al.,* 2010). The methanol (MeOH) and water extracts of *Spirulina plantensis* were active against Adenaovirus type 40 and reduces 50% and 23% respectively. The nontoxic concentrations for all the extracts were 2 mg/ml (Sayda M. Abdo *et al.,*2012).

Water and nonpolar extracts of *Spirulina platensis* exhibited antiviral activity against HSV -1 (Chirasuwan, 2009).The water extract of algae displays antiviral activity due to presence of sulphated polysaccharides (Sayda *et al.,* 2010 ; Singh *et al.,* 2011). The microalgae like *ankistrodesmus convolutus, Synechococcus elongates and Spirulina platensis* are active against Epstein Barr virus (EBV) in three Burkitt's lymphoma cell lines and a source of antiviral compound (Kok *et al.,* 2011). Spirulina is active against HIV-1, HIV -2, influenza virus and HSV (Singh, 2011, Sayda *et al.,*2012). The water and methonol extracts of *Spirulina* could be good source as antiviral agent (Sayda *et al*., 2012). Viral absorption / penetration and replication stages of progeny of several enveloped viruses blocked by the substances of *Spirulina platensis,* including HCV. Dietary supplementation of *spirulina* activates production of Interferon gamma (IFN - gamma), cytokines, NK cells, B cells and T cells of immune cells and thus stimulates immune response (Yakoot & Salem, 2012). The biochemical composition and topology of selected microalgae was reviewed by (Olivier Pignolet *et al.,* 2013). The protective effects blue-green algae against viral and bacterial infections, cancer, diabetes, allergies, hyperlipidemia and inflammation documented well (Ku et al., 2013).

To assess potential health concerns *of Spirulina* the Dietary Supplements Information Expert Committee (DSI-EC) reviewed from the recent information, analyzed adverse effects reports. The DSI-EC concluded that *Spirulina* supplement is accepted as a safe food except in some conditions (Belay, 2002; Marles *et al.,* 2011; Theodore G. Sotiroudis and Georgios T. Sotiroudis, 2013).

From the above studies it is clear that in an alternative approaches, therapeutic use of algae and its products may contribute more to combat diseases caused by viruses.

CONCLUSIONS

Spirulina plantensis contains many nutrients including protein pigments such as phycocyanin, allophycocyanin, phycoerythrin, chlorophyll and carotenoids, protein, aminoacids sulfolipids, polysaccharides, minerals and vitamins. Many

research results say that *Spirulina plantensis* can be used as therapeutic agent and also as antiviral agent.

ACKNOWLEDGEMENTS

I thank **UGC, New Delhi** for providing financial assistance and was supported by the UGC Major Research Project**.** I thank **BADRI. KAMESHWAR RAO, USA, Prof. D.V.R. Sai Gopal,** Head Department of Virology, S.V.University and **Prof. S.D.S. Murthy**, Head, Department of Biochemistry, S.V.University, Tirupati, AP, India.

Note: I thank all the authors quoted in this article for their contribution and their research on novel antiviral drug development.

REFERENCES

1. Armida Zúñiga-Estrada, Blanca Lilia Barrón, J Martín Torres-Valencia, & Germán Chamorro- Cevallos (2007).Spirulina as an Antiviral Agent, 227-242.
2. Abdulmumin A. Nuhu (2013). *Spirulina (Arthrospira)***:** An Important Source of Nutritional and Medicinal Compounds, Journal of Marine Biology, Volume 2013, Article ID 325636, 8.
3. Amha Belay (2002), The potential applications of *Spirulina* (Arthrospira) as a nutritional and therapeutic supplement. *J. American. Neutraceutical Association* (Spring, 2002), 5(2), 27-48.
4. Archana Kulshestha, Anish Zacharia, J., Urmila Jarouliya, Pratiksha Bhadauriya, Prasad.G.B. K.S. & Bisen, P.S. (2008). Spirulina in health care management, *Curr. Pharm. Biotechnol*, 9 (5): 400-405.
5. Ayehunie S, Belay A, Baba T.W & Ruprecht R.M. (1998). Inhibition of HIV-1 replication by an aqueous extract of *Spirulina platensis (Arthrospira platensis). J Acquired Immune Deficiency Syndromes and Human Retrovirology.*18:7-12.
6. Belay, A. (2008) *Spirulina in Human Nutrition and Health*, M. E. Gershwin, A. Belay, Eds.,CRC Press, Taylor and Francis Group, Boca Raton, London, New York. p. 1.
7. Blinkova, L.P, Gorobets, O.B & Baturo, A.P (2001). Biological activity of *Spirulina*, Zh Mikrobiol Epidemiol Immunobiol, 2, 114 – 118.
8. Capelli, B. & Cysewski, G. R. (2010) *Nutrafoods* 9, 19.
9. Chirasuwan Nattayaporn, Ratana Chaiklahan, Prasat Kittakoop, Wanlop Chanasattru, Marasri Ruengjitchatchawalya, Morakot Tanticharoen & Boosya Bunnag (2009). Anti HSV-1 activity of sulphoquinovosyl diacylglycerol isolated from *Spirulina platensis, ScienceAsia* 35: 137–141.
10. Chirasuwan, N, Chaiklahan, R, Ruengjitchatchawalya, M, Bunnag, B, & Tanticharoen, M. (2007) Anti HSV-1 activity of *Spirulina platensis* polysaccharide. *Kasetsart J (Nat Sci)* 41, 311–8.
11. Cleaveland, S., Laurenson, M.K. & Taylor, L.H. (2001). Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Phil. Trans. R. Soc. Lond. B.* 356, 1411, 991-999.
12. Clifton, R. & Lacy, M.D. (2007). New Developments in Biothreats and Biosecurity.
13. D. von der Weid (2000). *Malnutrition: a silent massacre*, Antenna Technologies,

    http://www.antenna.ch/en/documents/MonoUK1.pdf (accessed on 7/12/2012).

14. Deng, F., Lu, J.J., Liu, H.Y., Lin, L.P., Ding, J. & Zhang, J.S. (2011). Synthesis and antitumor activity of novel salvicine analogues. *Chin Chem Lett.*,**22:** 25-28.

15. Deng, R. & Chow, T.-J. (2010) *Cardiovasc. Ther*. 28, e33

16. Falquet, J. (1997). *The Nutritional Aspects of Spirulina*, Antenna Technologies,

    http://antenna.ch/en/documents/AspectNut_UK.pdf (accessed on 7 Dec., 2012)

17. FAO Corporate Document Repository, *The Use of Saline Waters for Crop Production*,

    http://www.fao.org/docrep/T0667E/t0667e04.htm#TopOfPage (accessed on 7/12/2012)

18. FAO, *How to Feed the World in 2050, High-Level Expert Forum, Global agriculture towards 2050, 2009*, http://www.fao.org/fileadmin/templates/wsfs/docs/Issues_papers/

19. Feldmann, S. C., Reynaldi, S., Stortz, C. A., Cerezo, A. S. & Damont, E. B. (1999). Antiviralproperties of fucoidan fractions from Leathesia difformis. Phytomedicine 6, 335–340.

20. Fineberg, H.V & Wilson, M.E. (2010). Emerging Infectious Diseases, International Risk Governance Council, 2010 - report "The Emergence of Risks: Contributing Factors" and is part of phase 1of IRGC's project on Emerging Risks.

21. Gardner, G. & Halwell, B. (2000). *Overfed and Underfed: The Global Epidemic of Malnutrition*, Worldwatch paper 150, J. A. Petersen, Ed., World watch Institute.

22. Gorobets, O.B, Blinkova, L.P & Baturo, A.P. (2002). Action of Spirulina platensis on bacterial viruses, *Zh Mikrobiol Epidemiol Immunobiol.* (6):18-21.

23. Gustafson, K.R, Cardellina, J.H II, Fuller, R.W, WeslowO.S, Kiser, R.F, Snader, K.M, Paterson, G.M, & Boyd, M.R (1989). AIDS-antiviral sulfolipids from cyanobacteria (blue green algae). *J Natl Cancer Inst*81: 1254-1258.

24. Hayashi K, Hayashi T, & Kojima I. (1996). A natural sulfated polysaccharide, calcium spirulan, isolated from *Spirulina platensis*: *in vitro* and *ex vivo* evaluation of anti-*Herpes simplex* virus and anti-human immunodeficiency virus activities. *AIDS Research and Human Retroviruses*, 12:1463-1471.

25. Hayashi, K. Hayashi, T & Morita, N. (1993). An extract from *Spirulina platensis* is a selective inhibitor of Herpes simplex virus type 1 penetration into HeLa cells. *Phytother Res*.,7:76-80.

26. Hayashi, T., Hayashi, K Maedaa M, & Kojima, I. (1996). Calcium spirulan, an inhibitor of enveloped virus replication, from a bluegreen alga *Spirulina platensis. J Nat Prod*.59:83-87.

27. Hayashi T. (2008). Studies on evaluation of natural products for antiviral effects and their applications, *Yakugaku Zasshi*. 128(1):61-79.

28. Hemtanon, P., Direkbusarakom, S., Bunyawiwat, V. & Tantitakoon. O. (2005). Antiviral and Antibacterial Substances from *Spiriluna platensis* to combat White Spot Syndrome Virus and *Vibrio harveyi*. *In* P. Walker, R. Lester and M.G. Bondad- Reantaso (eds). Diseases in Asian Aquaculture V, pp. 525-534. Fish Health Section, Asian Fisheries Society, Manila.

29. Henrikson R. (2009). *Earth Food Spirulina*, Ronore Enterprises, Inc., Hana, Maui, Hawaii, http://www.*Spirulina*source.com/PDF.cfm/EarthFood*Spirulina*.pdf (accessed on 7 Dec., 2012).

30. Hernández-Corona A, Nieves I, Meckes M, Chamorro G, & Barron BL. (2002). Antiviral activity of Spirulina maxima against herpes simplex virus type 2. *Antiviral Res.*56 (3):279-85.

31. ICTV: International Committee on Taxonomy of Viruses. www.ictvdb.org/Ictv/ICTVdBsearch.htm (accessed 16 August 2010).

32. Jo Halliday, Chris Daborn, Harriet Auty, Zacharia Mtema, Tiziana Lembo, Barend M. deC. Bronsvoort, Ian Handel, Darryn Knobel, Katie Hampson & Sarah Cleaveland (2012). Bringing together emerging and endemic zoonoses surveillance: shared challenges and a common solution, *Phil. Trans. R. Soc. B.*, 367, 1604, 2872-2880.

33. Karkos, P. D., Leong, S. C., Karkos, C. D., Sivaji, N. & Assimakopoulos, D. A. (2008). *Spirulina* in Clinical Practice: Evidence-Based Human Applications, Hindawi publishing corporation, vol: 2011, 1-4. *Evid.-Based Compl. Alt.* doi:10.1093/ecam/nen058.

34. Kazuya I.P.J. Hidari, Tomoko Abe & Takashi Suzuki (2013).Crabohydrate-Related Inhibitors of Dengue Virus Entry, *Viruses*, 5,605-618. doi:10.3390/v5020605

35. Kenji, L. K. et al. (2005).Isolation of an antiviral polysacharide, nostoflan, from a terrestrial cyanobacteium, Nostoc flagilliforme. J. Nat. Prod. 68, 1037–1041.

36. Kok, Y.Y, Chu, W. L, Phang, S.M, Mohamed, S.M, Naidu, R, Lai, P.J, Ling, S.N, Mak, J.W, Lim, P.K, Balraj, P & Khoo, A.S. (2011). Inhibitory activities of microalgal extracts against Epstein – Barr virus DNA release from lymphoblastoid cells, Zhejiang. Univ.Sci B, 12 (5), 335-45.

37. Ku, C.S, Pham, T.X, Park, Y, Kim, B, Shin, M.S, Kang, I & Lee, J (2013). Edible blue-green algae reduce the production of pro-inflammatory cytokines by inhibiting NF-kappaB pathway in macrophages and splenocytes. Biochim Biophys Acta, 1830(4):2981–2988.

38. Lau, A.F., Sied leck J, Anleitner J, Patterson GM, Caplan FR, & Moore RE (1993). Inhibition of reverse transcriptase activity by extracts of cultured blue green algae (cyanophyta), Planta Med., 59: 148 -51.

39. Lee RE (2008) Phycology, 4th edn. Cambridge University Press, Cambridge.

40. Lee, J.B, Srisomporn.P, Hayashi.K, Tanaka.T, Sankawa. U & Hayashi,T. (2001).Effects of structural modification of calcium spirulina, a sulphated polysaccharides from *Spirulina platensis*, on antiviral activity, Chem Pharm Bull (Tokyo), 49 (1), 108 – 110.

41. Louise H. Taylor, Sophia M. Latham & Mark E.J. woolhouse (2001). Risk factors for human disease emergence, *Phil. Trans. R. Soc. Lond. B.* 356, 1411, 983-989.

42. Mark Woolhouse, Fiona Scott, Zoe Hudson, Richard Howey & Margo Chase-Topping (2012) Human viruses: discovery and emergence, *Phil. Trans. R. Soc*. B, 367, 2864–2871.

43. Marles, R. J. Barrett, M. L. Barnes, J. Chavez, M. L. Gardiner, P. Ko, R., Mahady, G. B., Low Dog, T., Sarma, N. D., Giancaspro, G. I., Sharaf, M. & Griffiths, (2011). J. *Crit. Rev. Food Sci. Nutr.* 51, 593.

44. McCarty, M. F. (2007) *J. Med. Food* 10, 566

45. McCarty, M. F. Hendler, S. S., Rorvik, D. M. & Inoguchi, T. (2010) US 2010/0172971 A1.

46. Medina-Jaritz, N. B., Perez-Solis, D. R., S. L. Ruiloba de Leon F. and Olvera-Ramírez, R. (2011). Antimicrobial activity of aqueous and methanolic extracts from *Arthrospira maxima*, Science against microbial pathogens: communicating current research and technological advances, A_. M__éndez-_V_ilas _(E_d_.),©FORMATEX 2011, 1267- 1271.

47. Mostafa Yakoot & Amel Salem (2012).Spirulina platensis versus silymarin in the treatment of chronic hepatitis C virus infection.A pilot randomized, comparative clinical trial BMC Gastroenterology, 12:32.

48. Olivier Pignolet, Se´bastien Jubeau, Carlos Vaca-Garcia & Philippe Michaud (2013). Highly valuable microalgae: biochemical and topological aspects, J Ind Microbiol Biotechnol (2013) 40:781–796.

49. Patterson GML, Baker KK, Baldwin CL, Bolis CM, Caplan FR, Larson LK, Levine IA, Moore RE, Nelson CS, Tschappat KD & Tuang. G. (1993). Antiviral activity of cultured blue-green algae (Cyanophyta). *J Phycol*.29:125-130.

50. Rahul Kunwar Singh, Shree Prakash Tiwari, Ashwani K Rai & Tribhuban M Mohapatra (2011). Cyanobacteria: an emerging source for drug discovery, *The Journal of Antibiotics*, 64, 401–412.

51. Rakhi Bajpai Dixit & M. R. Suseela (2013). Cyanobacteria: potential candidates for drug discovery, Antonie van Leeuwenhoek, 103: 947–961. DOI 10.1007/s10482-013-9898-0

52. Sayda M. Abda, Mona H. Hetta, Waleed M. EI- Senousy, Rawheya, A. Salah EI Din & Gamila H. Ali (2012). Antiviral activity of fresh water algae, J. Applied pharmaceutical sciences, 2 (2), 21-25.

53. Shih, S.R, Tsai, K.N, Li,Y.S, Chueh,C.C & Chan, E.C (2003). Inhibition of enterovirus 71 – induced apoptosis by allophycocyanin isolated from a blue – green alga Spirulina platensis, J.Med. Virol., 70 (1), 119 – 25.

54. Simpore J, Zongo F, Kabore F, Dansou D, Bere A, Nikiema JB, Pignatelli S, Biondi DM, Ruberto G & Musumeci S. (2005).Nutrition rehabilitation of HIV-infected and HIV-negative undernourished children utilizing spirulina. Ann Nutr Metab. 49(6):373-80.

55. Singh, R.K, Tiwari, S.P, Rai A.K & Mohapatra, T.M. (2011) Cyanobacteria: an emerging source for drug discovery. J Antibiot 64:401–412.

56. Soheili, M. & Khosravi-Darani, K. (2011). *Curr. Nutr. Food Sci*. **7,** 279.

57. Theodore G. Sotiroudis & Georgios T. Sotiroudis (2013).Health aspects of *Spirulina* (*Arthrospira*) microalga food Supplement, *J. Serb. Chem. Soc. 78* (3) 395–405.

58. UNICEF, *Vitamin and Mineral Deficiency, A global damage assessment report*,

    http://www.micronutrient.org/CMFiles/PubLib/Report-67-VMD-A-Global-Damage-Assessment-Report1KSB-3242008-9634.pdf (accessed on 7/12/2012).

59. WHO (1996).The World Health Report, 1996: Fighting disease, fostering development, Geneva,

60. WHO 2013.

61. Witvrouw M & De Clercq E (1997) Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. Gen Pharmacol 29:247–511.

62. www. Google.com

63. Zainuddin, E. N. *et al.* (2007).Cyclic depsipeptides, ichthyopeptins A and B, from Microcystis ichthyoblabe. J. Nat. Prod. 70, 1084–1088.